SCUTELLOSPORA CASTANEA, A NEWLY DESCRIBED ARBUSCULAR MYCORRHIZAL FUNGUS.

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ABSTRACT - *Scutellospora castanea*, a newly described fungus forming arbuscular mycorrhizas was isolated from soil beneath *Lathyrus sylvestris* in France. It was established in pot culture with *Allium porrum* and has been maintained for more than ten years on various host plants. The species is characterised by its shiny, more or less globose, chestnut-coloured spores which are on average 300 µm in diameter and which possess a brittle outer wall group enclosing a flexible inner wall group on which is formed an indistinct germination shield. It can be distinguished from *S. arenicola* mainly by the lack of an amorphous spore wall, and from *S. erythropa* on the basis of spore size and wall structure.

RÉSUMÉ - Une nouvelle espèce de champignon, *Scutellospora castanea* formant des mycorhizes arbusculaires a été isolée d'un talus près de Pau en France. La culture de ce champignon a été établie sur *Allium porrum* en pot et ensuite maintenue pendant plus de dix ans sur différentes plante-hôtes. Cette espèce fongique forme des spores d'environ 300 µm de diamètre qui se caractérisent par une surface lisse et brillante, une forme plus ou moins globuleuse et une couleur châtaigne. Elles possèdent une paroi externe cassante et une paroi interne flexible sur laquelle se forme une plaque de germination mal définie. *Scutellospora castanea* se différencie de *S. arenicola* principalement par l'absence de paroi amorphe, et de *S. erythropa* par la taille et la structure pariétale des spores.

KEY WORDS Arbuscular mycorrhizas, *Scutellospora castanea*, Gigasporaceae, Glomales

INTRODUCTION

A sample of soil (pH 7.7 in deionised water; Olsen-extractable P 11 ppm), taken from beneath *Lathyrus sylvestris* L. on a roadside verge near Pau, Toulouse in 1983, was subjected to wet sieving and decanting (Gerdemann & Nicolson, 1963). Amongst the spores of members of the Glomales obtained in this way were those of an apparently undescribed member of the arbuscular-mycorrhizal genus *Scutellospora*. Some of these spores were used to inoculate seedlings of *Trifolium pratense* L. growing in irradiated clay loam (Eposisses) soil of pH 7.4 (in deionised water). The resulting pot cultures produced arbuscular mycorrhizas and abundant spores after a few

months. The isolate thus obtained has since been repeatedly sub-cultured on several hosts including *Allium cepa* L., *A. porrum* L., *Plantago major* L. and *P. lanceolata* L.

MATERIAL AND METHODS

Spores for the species description were extracted by a centrifugation-flotation technique (Walker et al., 1982) or by shaking pot culture substrate in water and decanting the supernatant onto a 160 µm sieve. The spores were then suspended in a dish of water and examined first under a dissecting microscope with reflected light and later under a compound microscope with brightfield illumination or with Nomarski Differential Interference Contrast. Roots were removed from pot cultures, subjected to clearing and staining (Phillips & Hayman, 1970), and examined under a compound microscope for the establishment of mycorrhizas and associated extra-matrical mycelium.

In an attempt to standardise colour matching, spores suspended in water were illuminated with light from a quartz iodine fibre-optic source at a colour temperature of 3200 K and examined under a dissecting microscope. Their colours were then compared with those on a fungal colour chart (Anon, 1969) illuminated simultaneously by a split fibre-optic from the same source. For more detailed examination, spores were mounted on microscope slides in water (Spain, 1990), or polyvinyl alcohol lacto glycerol (PVLG) (Koske & Tessier, 1983). Colour matching of structures viewed with transmitted light under a compound microscope is more difficult than with a dissecting microscope, so for such observations colours (for example, of individual walls in the descriptions) were not precisely matched to a chart, and only generalised colour descriptions are used.

Type material, consisting of a holotype collection on a microscope slide, and isotype material on microscope slides, in dried soil, and preserved in both 5% formaldehyde solution and 0.025% NaN_3, has been lodged at the herbarium of the Royal Botanic Garden, Edinburgh (E). Isotype material has also been lodged at Oregon State University (OSC). The species description is based on the style adopted by Gerdemann & Trappe (1974), with modifications of spore wall terminology as suggested by Walker (1983). Some of the terminology of Spain et al. (1989) has been used to describe the hypha upon which the spore is borne.

SPECIES DESCRIPTION

***Scutellospora castanea* Walker sp. nov.** Figs. 1-16

Sporae in solo singillatim efformatae, ad basis bulbosa terminales vel laterales, globosae vel subglobosae, raro ovoideae vel obovoideae, 169-369 x 176-372 µm, juventute candidae, opacae, maturitate ochraceae vel avellaneae, crescenter vacuolatae translucentes. Tunicae sporae stratis quatuor in turmis duabus. Turma externa stratis duobus: stratum extimum laeve, nitidum, brunneum, 2-4 µm crassum, stratum internum lamellatum, hyalinum vel luteolum, 10-25 (-35) µm crassum. Turma interna stratis duobus: stratum extimum tenuissimum, hyalinum, flexile, minus quam 1 µm crassum, stratum internum flexile, 1-2 µm crassum. Turma interna scutello germinationis ovoideo complexo, hyalino vel luteolissimo, usque ad 208 x 181 µm. Basis bulbosa 38-51 µm crassa. Cellulae auxiliares pallide avellaneae vell brunneolae, singulares vel fasciculatae, prominentus nodosis, obtusis.

Spores borne singly in the soil, terminally or laterally on a bulbous base, globose to subglobose, rarely ovoid or obovoid, 169-369 x 176-372 µm, pure white and

opaque when immature, shading through ochraceous (8G) to sienna (11) and becoming increasingly vacuolate and translucent with maturity (Fig. 1). **Spore wall** of 4 walls organised in 2 groups (Fig. 10). Outer group A of 2 walls (Fig. 3): wall 1 loosely adherent, smooth, shiny, brown, unit, 2-4 µm thick; wall 2 hyaline to pale yellow, laminate, 10-25 (-35) µm thick. Inner group B of two walls (Fig. 5 & 9): wall 3, very thin, hyaline, flexible (membranous), less than 1 µm thick, closely adherent to wall 4; wall 4 flexible (membranous), 1(-2)mm thick. **Germination shield** on wall group B in mature spores hyaline to very pale yellow, with complex infolding of the edges, up to 208 x 181mm (Fig. 7). **Bulbous base** 38-51 mm wide (Fig. 2). **Auxiliary cells** pale yellow brown to pale brown, single or clustered, with blunt, knobby projections.

Reaction to PVLG/Melzer's reagent (5:1 v/v) is variable, probably depending on spore maturity. In wall group A, wall 1 becomes a deeper brown and wall 2 occasionally becomes pink or red, but usually acquires a deeper yellow colour (Fig. 4). In wall group B, neither wall reacts to Melzer's reagent, but the thin-walled, complex germination shield sometimes darkens to become a pale brownish yellow. This structure is usually difficult to observe due to it being thin, a pale colour and the masking caused by the brown pigmentation in wall group A, but it can be visualised by bleaching spores (Fig. 7).

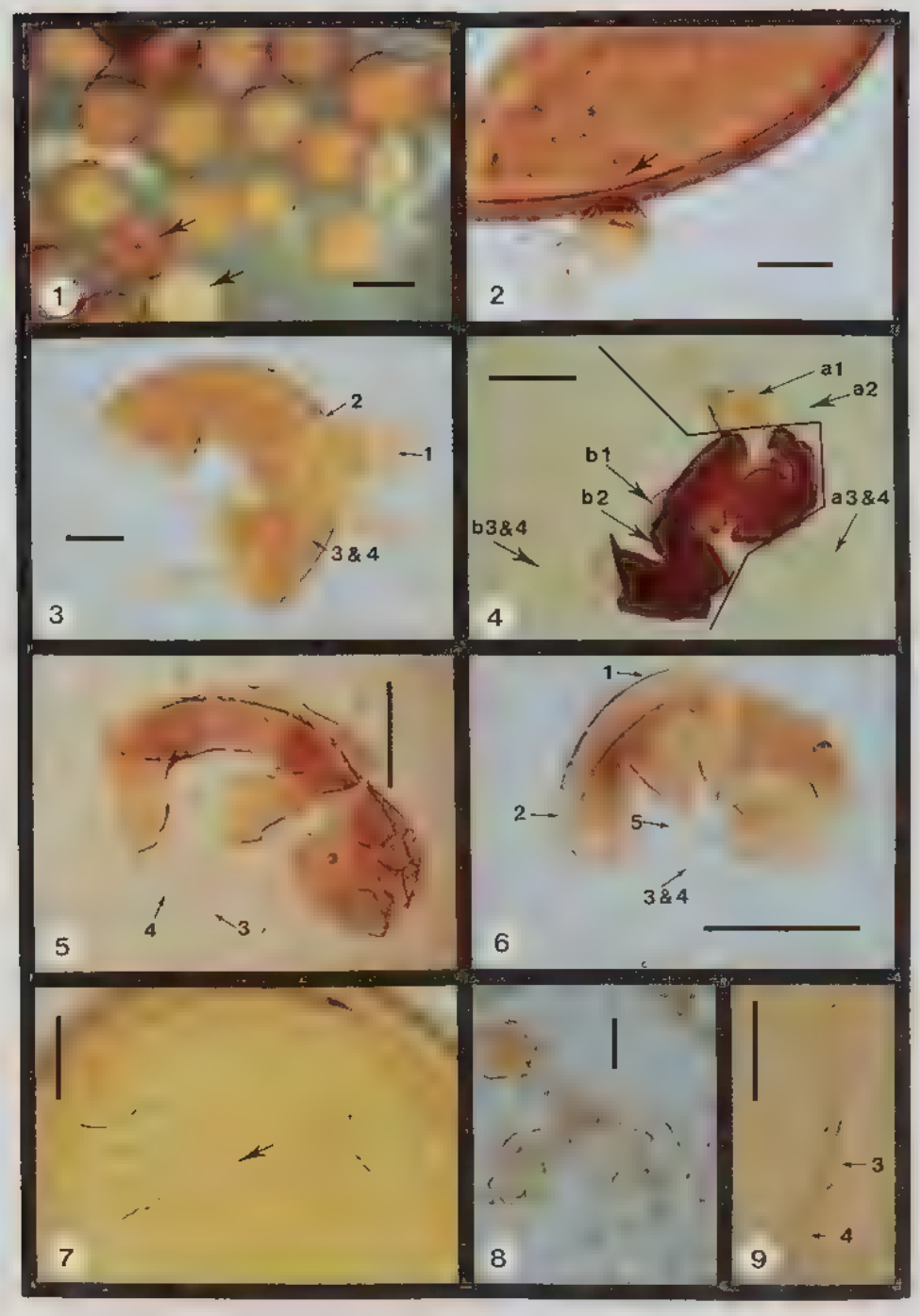
The bulbous base (= bulbous suspensor-like cell of Gerdemann & Trappe (1974) or sporogenous cell of Spain et al. (1989)), which is concolorous with the outer spore wall layers (Fig. 2) and can have up to five hyphal projections, is borne terminally on a septate sporophore 14-20 µm wide formed from a broad, pale yellow-brown, coenocytic hypha approximately 7-15 µm wide. The bulbous base detaches particularly easily in this species, and is missing from a large proportion of spores extracted by processes involving sieving.

S. castanea forms endomycorrhizas (Figs 11-16) with hyphae developing appressoria (Fig. 12), H-connections (Abbott, 1982) (Figs 13 & 15), hyphal coils (Fig. 14) and arbuscules (Fig. 16) but without vesicles.

DISCUSSION

There are only two other described species of *Scutellospora* possessing brown spores with a smooth, shiny surface. These are *S. erythropa* Koske & Walker and *S. arenicola* Koske & Halvorsen. Spores of the former have a greater size range with larger maxima (170-551 x 205-660 compared with 169-369 x 176-372 µm), are much darker in colour, and have much more opaque outer walls than those of *S. castanea*. They also have a different wall structure and a much more distinct and robust germination shield (Koske & Walker 1984). *Scutellospora arenicola* and *S. castanea* spores are similar in colour, have similar size ranges (160-360 x 120-310 µm for *S. arenicola*) and possess similarly indistinct germination shields. However, the wall structures of *S. arenicola* and *S. castanea* differ in one important respect. The former has one wall more than the latter - an amorphous innermost wall (Fig. 6) that reacts distinctively to Melzer's reagent to give a purple reaction (Koske & Halvorsen, 1989). Differences of this nature are considered to result from major evolutionary change attributable to phylogenetic grouping such as species (Morton et al., 1992).

Unusually for a species of *Scutellospora*, wall 1 (the outer, unit wall) can be detached with relative ease from wall 2 as the spore is crushed. The wall does not detach completely, however, but cracks radially, and then separates in parts (Fig. 3). Occasional spores can be seen to have lost patches of the outer wall even under the dis-



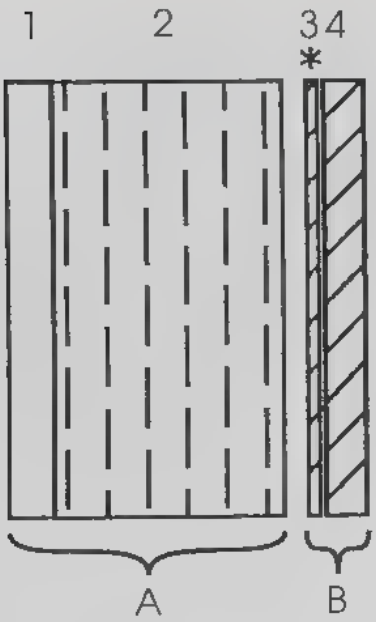


Fig 10 - Murograph of *S castanea*

Fig 1 - Freshly extracted spores suspended in water and illuminated by incident light Immature (lower arrow) and over mature (upper arrow) spores are indicated The vacuolate nature of the spore contents in mature, healthy spores can be seen in the majority of the remaining spores. Scale bar 250 µm

Fig 2 Detail of spore showing the bulbous base The point of connection of outer and inner wall groups is arrowed. Scale bar 50 µm

Fig 3 Crushed spore mounted in PVLG showing walls 1 and 2 in group A separating, and walls 3 and 4 in group B remaining together Scale bar 100 µm

Fig 4 This preparation shows two heavily crushed spores (labelled a and b) Spore 'a' (right of dividing line) was crushed in PVLG Spore b' (left of dividing line) was crushed in PVLG with Melzer's reagent The spores were then re-mounted in PVLG Walls 1 & 2 have reacted in the Melzer's reagent (arrowed b1 and b2), whereas walls 3 and 4 have not Scale bar 200 µm

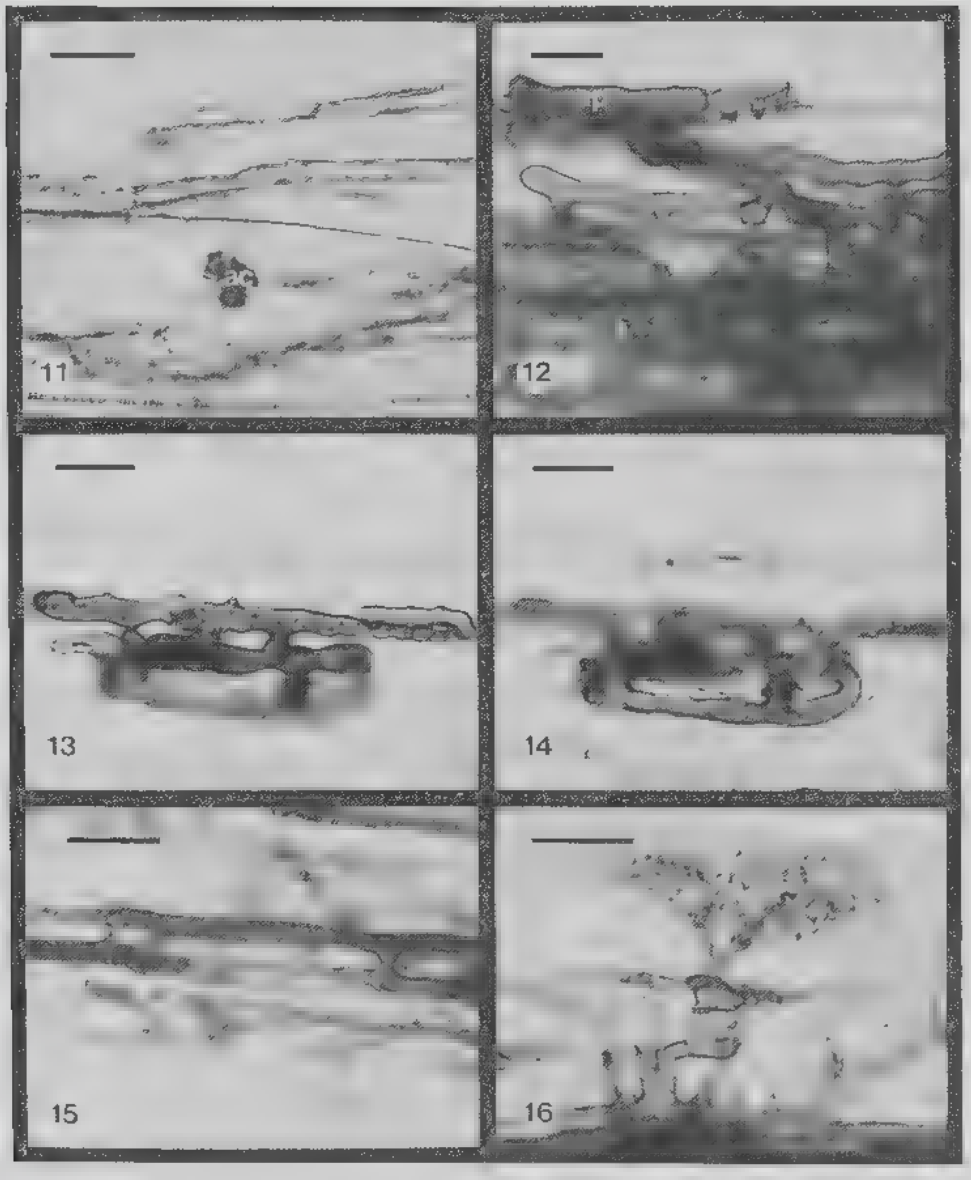
Fig 5 - A spore crushed heavily in PVLG Walls 3 and 4 in the inner wall group are arrowed and numbered Scale bar 250 µm

Fig 6 - A similar preparation to that in Fig 5, but from the isotype material of *Scutellospora arenicola*, showing the additional wall (wall 5). Scale bar 250 µm

Fig 7 - A spore bleached with undiluted domestic bleach to reveal the germination shield The lumen at the point of origin is arrowed. Scale bar 50 µm

Fig 8 - Knobby auxiliary cells of *Scutellospora castanea*. Scale bar 25 µm

Fig 9 - Detail of the inner wall group of *S castanea* (walls 3 and 4 arrowed) Scale bar 100 µm



Figs 11-16 - Mycorrhiza of *Allium porrum* and *Scutellospora castanea*

Fig 11 Cleared and stained root squash showing the hyphal elements stained with trypan blue. The stele is at the bottom of the photograph. A small cluster of auxiliary cells is seen on the root surface (ac) Fig. 12 - Lateral view of an appressorium-like structure (ap) with resultant coarse, intra- and inter-cellular hyphal elements colonising the root. Fig 13 - Plan view of an appressorium-like structure on the root surface. Fig. 14 - Lower focus of Fig 13, showing the hyphal coil occupying a cell beneath the appressorium-like structure Fig 15 - Inter cortical hyphae, showing H-connections Fig. 16 - Arbuscule in a cortical cell

secting microscope at magnifications as low as 30x This phenomenon was also reported for spores of *S. arenicola* (Koske & Halvorsen, 1989)

Stimulated by the work of Spain (1990), fresh specimens were examined in water No great differences were noted in apparent wall structure when compared with PVLG-mounted specimens, except that in the latter, wall 3 was a little easier to see On some specimens, both in water and PVLG, walls 3 and 4 were so tightly adherent, that they were only distinguishable under magnifications of greater than 500 X This was particularly the case for spores that had been preserved in 0 025% sodium azide solution The laminae in wall 2 can be so fine that it appears superficially to be a unit wall, though splits forming along a lamina in some spores gave a clue to its true nature, and when heavily crushed, the laminae can be seen as steps where they have broken differentially The bulbous structure at the base of the spore is not a cell, but is continuous with the outer walls of the spore We have therefore not used the terms bulbous suspensor like cell (Gerdemann & Trappe, 1974) or 'sporogenous cell (Spain et al , 1989) The walls can best be understood through their development series In white spores, the earliest development is the production of an apparently 2 layered wall (walls 1 & 2 in group A), formed directly by further development of the walls of the suspensor-like cell In some specimens, there seems to be a hyaline layer between walls 1 and 2, but this is not always observable, and has not been considered to be a separate wall A similar wall or wall layer was described as being present in *S. gregaria* Koske & Walker, though in that species it was considered to be a separate laminated wall (Koske & Walker, 1985) As the spores darken with age, both the outermost wall and the contents change colour After the spores have started to change colour, the flexible walls develop, first by formation of the extremely thin flexible membranous wall (wall 3), and then by the production of the innermost flexible wall (wall 4) The thin, membranous wall (wall 3) forms an endosporangium like structure enclosing the second flexible wall that forms a single entity similar to a sporangiospore

Unlike most *Scutellospora* spp., the flexible walls of *S. castanea* do not readily separate from the outer wall group Consequently, on casual observation, even mature spores of this species can appear to belong to the genus *Gigaspora* This misapprehension is reinforced by the difficulties of seeing the germination shield because of the lack of either colour or thickening at its edges, and by the presence in some specimens of a layer that appears to be similar to the germinal wall of Spain et al ,1989 Because this is detectable only on some spores, we have assumed it merely to be an innermost lamina of wall 2, but ultrastructural study may prove this interpretation to be erroneous

Scutellospora castanea possesses the hyphal bridging or wound healing described by Gerdemann (1955) However, it should be noted that this phenomenon does not always result from wounding but can occur on lengths of apparently healthy hyphae as a result of branching and later anastomosis The hyphae may also form mycelial strands by repeated branching and anastomosis both within a hypha and between adjacent hypha.

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